

CLAIMS

1 1. A method for detecting an unbound form of a first member of a binding pair, the
2 binding pair comprising a first and second member, each member bindable to the
3 other, the method comprising the steps of:

4 (a) providing a first particle bound to the second member;

5 (b) reacting the first particle bound to the second
6 member with a sample, thereby forming a first complex between
7 the second member bound to the first particle and unbound first
8 member present in said sample;

9 (c) providing a second particle bound to a third member, the third
10 member being different from the second member and being
11 capable of binding to the first member;

12 (d) reacting the second particle bound to the third
13 member to the sample, thereby forming a second complex between
14 the third member bound to the second particle and the first
15 complex; and

16 (e) detecting any second complex formed.

1 2. The method of claim 1, wherein the third member is an antibody which
2 specifically binds to the first member.

1 3. The method of claim 1, wherein the first and/or second particle is latex.

1 4. The method of claim 1, wherein the second complex is detected by measuring an 10^3
2 increase in the turbidity of the sample.

1 5. The method of claim 1, wherein steps (a) through (d) are performed sequentially.

1 6. The method of claim 1, wherein steps (a) through (d) are performed 10^3
2 simultaneously.

1 7. The method of claim 1, wherein the amount of second complex formed is
2 quantitated.

1 8. The method of claim 1, wherein the first member is protein S. 10^3

1 9. The method of claim 1, wherein the second member is C4b-binding protein 10^3
2 (C4BP).

*10^3 obvious
modification
of prior
art.*

1 10. The method of claim 1, wherein the sample is selected from the group consisting
2 of blood, plasma, serum, saliva, CSF, urine, culture media, a cell suspension, a
3 buffer and an artificially prepared fluid containing the first member.

1 11. The method of claim 1, wherein the second member binds to the first member at a
2 single binding site.

1 12. The method of claim 11, wherein the third member binds to the first member at a
2 single binding site which is different from the single binding site to which the
3 second member binds.

1 13. The method of claim 1, wherein step (b) is performed within 0 to about 180
2 seconds.

1 14. The method of claim 1, wherein the molar ratio of third member to second
2 member is between about 2 and 20.

1 15. The method of claim 1, wherein the molar ratio of the third member to second
2 member is between about 5 and 10.

1 16. The method of claim 1, wherein the amount of third member is higher than the
2 amount of free first member in the sample.

1 17. The method of claim 1, wherein the molar ratio of third member is between about
2 10 and 40 times the amount of free first member in the sample.

1 18. A composition for detecting an unbound form of a first member of a binding pair,
2 the binding pair comprising a first and second member, each member bindable to the
3 other, the composition comprising:
4 a first particle bound to the second member;
5 a second particle bound to a third member, the third member being
6 different from the second member and capable of binding to the first
7 member at a binding site different from the second member.

1 19. The composition of claim 18, wherein the first member is protein S and the second member is C4BP.

1 20. The composition of claim 18, wherein the third member is an antibody and the second member is not an antibody.

1 21. The composition of claim 18, wherein the second member comprises a single binding site for the first member.

Third
molar
ratios
General
principles

particle
with
a member
immobilized
in a...
chain

3 22. The composition of claim 21, wherein the third member binds to the first member 162
4 at a single binding site which is different from the single binding site to which the
5 second member binds.

1 23. A method for detecting an unbound form of a first member of a binding pair, the
2 binding pair comprising a first and second member, each member bindable to the
3 other, the method comprising the steps of:

4 (a) providing a first particle bound to the second member;
5 (b) reacting the first particle bound to the second member with a
6 sample, thereby forming a first complex between the second
7 member bound to the first particle and unbound first member
8 present in said sample;
9 (c) providing a second particle bound to the first member;
10 (d) reacting the second particle bound to the first member with the
11 sample, thereby forming a second complex between second
12 particle bound to the first member and first particle bound to
13 second member which is not already bound to the first member;
14 and
15 (e) detecting any second complex formed, wherein the amount of
16 second complex formed is inversely proportional to the amount of
17 unbound first member is the sample.

1 24. The method of claim 23, wherein the first and/or second particle is latex.

1 25. The method of claim 23, wherein the second complex is detected by measuring an
2 increase in the turbidity of the sample.

1 26. The method of claim 23, wherein the amount of second complex formed is
2 quantitated.

1 27. The method of claim 23, wherein the first member is protein S.

1 28. The method of claim 23, wherein the second member is C4BP.

1 29. The method of claim 23 wherein the sample is selected from the group consisting
2 of blood, plasma, serum, or an artificially prepared buffer containing the first
3 member.

1 30. A composition for detecting an unbound form of a first member of a binding pair
2 comprising a first and second member, each member bindable to the other, the
3 composition comprising:
4 a first particle bound to the second member; and
5 a second particle bound to the first member.

1 31. The composition of claim 30, wherein the first member is protein S and the
2 second member is C4BP.

1 32. A method for diagnosing thrombophilia comprising performing the method of
2 claim 8, and further comprising comparing the amount of second complex formed
3 to the amount of second complex formed in a sample derived from an individual
4 without thrombophilia.

1 33. A method for diagnosing thrombophilia comprising performing the method of
2 claim 27, and further comprising comparing the amount of second complex
3 formed to the amount of second complex formed in a sample derived from an
4 individual without thrombophilia.

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